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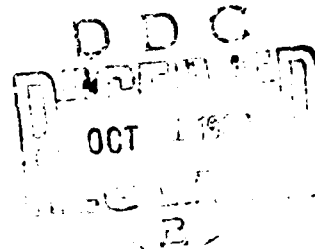
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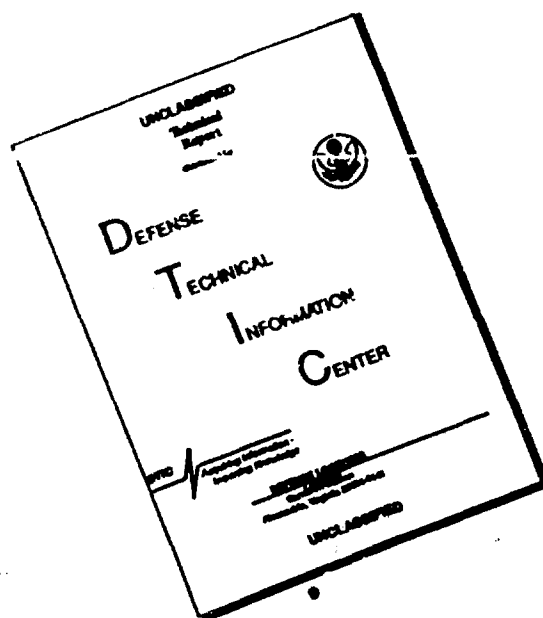
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IMMUNOGENIC AND ANTIGENIC ACTIVITY OF INACTIVATED TISSUE-  
CULTURE VACCINE FOR VIRUSES OF THE TICK-BORNE ENCEPHALITIS COMPLEX

[Following is the translation of an article by V. A. Zaklinskaya, D. K. Lvov, M. P. Chumakov and L. S. Levina, Institute for Poliomyelitis and Viral Encephalitises, AMN, USSR, Moscow, published in the Russian-language periodical Voprosy Virusologii (Problems of Virology), No. 6, 1965, pages 649-656. It was submitted on 9 July 1964. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

The existence of various viruses in the tick-borne encephalitis complex established the necessity for studying the effectiveness of a vaccine against one of the viruses in respect to the remaining representatives of the complex. The literature contains individual reports on this problem [5-9]. Serological investigations of human sera are limited to the identification of antibodies to the virus of Kyasanur Forest disease (KFD) in persons inoculated with vaccine against tick-borne encephalitis [6-7], and also antibodies to the viruses of tick-borne encephalitis and Langat after immunization with live Langat virus [1]. Considering the absence of systematic investigations in this direction, we studied the immunogenic and antigenic properties of tissue-culture vaccine in respect to all the presently known viruses of the tick-borne encephalitis antigen complex.

Materials and Methods

In the experiments we used industrial series of inactivated tissue-culture vaccine (with adsorbent) against tick-borne encephalitis, prepared at our institute. The Pan and Sofin strains were used as the seeding virus. The immunogenic properties of the vaccine were checked by testing the resistance of immunized (twofold subcutaneous immunization of 0.5 ml each with a one day interval) pure-line mice (BALE). The resistance index of the vaccinated animals was calculated based on the difference in the LD<sub>50</sub> among the test animals and control animals, infected intraperitoneally with the corresponding strain of virus.

The antigenic properties of the vaccine were determined by means of investigating the sera of 46 vaccinated (1x3 with 1-2, and 2-3 week intervals) and revaccinated (1 ml one year after vaccination) volunteers in the neutralization reaction (N) and the hemagglutination inhibition reaction (HI) with the appropriate viruses. Prior to the inoculations the volunteers were non-immune. The blood for the investigation was taken before and after 10 to 30 days after vaccination and revaccination.

The neutralization reaction was set up by means of intraperitoneal

titration on mice. If the viruses had a low peripheral activity, then the neutralization reaction was also set up intracerebrally.

The HI was set up by the generally accepted method (contact of antigens with sera at 4° for 18-20 hours, precipitation of 0.3% goose erythrocytes in a phosphate buffer at 4°). The sera were treated with a 25% suspension of kaolin on a borate buffer with pH 9.0 and a 30% suspension of goose erythrocytes on the same buffer. In the experiment we took 8 AU each of antigens. In the greatest majority of cases the optimum pH value was 6.4. During the process of work we constantly controlled the optimum pH value for each of the antigens (3-fold parallel titration of antigens with a set of various pH values).

In the work we used 8 strains of tick-borne encephalitis virus, isolated in the eastern and western regions of the USSR, in Czechoslovakia and Sweden, and also one each of the following virus strains: Louping ill, Omsk hemorrhagic fever, Kyasanur Forest disease, Langat, Powassan, and Negishi (Table 1).

**Immunogenic properties.** The results of investigating the immunogenic activity of the vaccine in the experiments on pure-bred mice are presented in Table 2. When analyzing the data it is necessary to take into consideration the different peripheral activity of the various viruses. The index of resistance of the vaccinated animals during the experiments with all the strains of the tick-borne encephalitis virus and with the Omsk hemorrhagic fever virus has a close value (6.3-4.2 lg) in contrast to the Powassan virus (2.9 lg). The small values for the index of resistance in the tests with the Langat, Negishi and Kyasanur Forest disease viruses, and especially the louping ill virus, are difficult to analyze due to the low virus titers in the controls in our experiments. The average values given are based on 2 experiments. The logarithm for the resistance index of the vaccinated animals is based on a record of 6.0 (during testing with the Absettarov strain).

Thus, the immunogenic properties of the vaccine were close in respect to all the utilized strains of the tick-borne encephalitis and Omsk hemorrhagic fever viruses and were sharply reduced in respect to the louping ill virus. A certain lowering in immunogenic activity is noted in respect to the viruses of Negishi, Kyasanur Forest disease, Powassan and Langat.

**Antigenic properties.** Data on the amount of sera (from inoculated volunteers), treated in the HI with the help of each of the 14 antigens, are presented in Table 3.

Figure 1 can be used to form an opinion on the frequency of detecting anti-hemagglutinins after vaccination. Ten days after vaccination, antibodies to all the strains of tick-borne encephalitis virus were detected with the same frequency (61-74%, an average of  $67 \pm 2.8\%$ ). The same

constancy was also shown by antibodies to the viruses of Omsk hemorrhagic fever ( $65 \pm 7.7\%$ ) and Langat ( $61 \pm 7.2\%$ ). Antibodies to the viruses of louping ill and Negishi were detected reliably less often ( $0.01 < P < 0.05$ ). From time to time it was possible to find antibodies to the viruses of Kyasanur Forest disease ( $9 \pm 5.2\%$ ) and Powassan ( $26 \pm 7.5\%$ ).

A correlation is noted between the frequency of appearance of antibodies and the level of antihemagglutinin titers to various viruses (Figure 2). The highest titers are observed in relation to all the strains of viruses of tick-borne encephalitis ( $3.0 \pm 0.1$  lg) and Omsk hemorrhagic fever ( $2.9 \pm 0.3$  lg). The titers to Langat virus were reliably lower ( $2.2 \pm 0.3$  lg;  $P < 0.001$ ). The lowest titers are detected for the viruses of Kyasanur Forest disease ( $0.4 \pm 0.2$  lg) and Powassan ( $0.9 \pm 0.2$  lg).

After revaccination, as it should have been expected, there was a significant increase in the number of persons with antibodies, and the antibody titers were higher (Figure 3). Antihemagglutinins to the tick-borne encephalitis virus appeared in 75-100% (an average of  $86 \pm 2.8\%$ ) of inoculated persons. To the viruses of louping ill, Omsk hemorrhagic fever, Langat and Negishi, antibodies were detected correspondingly in  $65 \pm 10$ ,  $63 \pm 8.8$ ,  $85 \pm 8$ , and  $55 \pm 9.2\%$  of the cases. As previously a positive HI was noted least of all with the antigens of Kyasanur Forest disease ( $52 \pm 10\%$ ) and Powassan ( $43 \pm 8.1\%$ ). Thirty days after revaccination the number of persons with antihemagglutinins was practically the same in the investigation of sera with the antigens of tick-borne encephalitis, Omsk hemorrhagic fever, Langat and Negishi. The number of persons with antibodies to the remaining viruses was reduced significantly (Figure 3).

As can be seen in Figure 4, the antibody titers after revaccination are lower to the viruses of louping ill ( $3.1 \pm 0.3$  lg;  $P < 0.001$ ), Omsk hemorrhagic fever ( $2.7 \pm 0.3$ ;  $P < 0.001$ ) and Negishi ( $2 \pm 0.3$ ;  $P < 0.001$ ) in comparison with the titers to the tick-borne encephalitis virus ( $4.2 \pm 0.2$  lg). As previously, the lowest titers are noted with the viruses of Kyasanur Forest disease and Powassan.

The data obtained correspond to the results of investigating the sera of inoculated persons in the neutralization reaction (Table 4).

The virus neutralizing activity of the sera of inoculated persons turned out to be the same in respect to all the strains of the tick-borne encephalitis virus, and somewhat lower to the viruses of Omsk hemorrhagic fever, louping ill and Negishi. Virus neutralizing antibodies to the viruses of Kyasanur forest disease, and especially Powassan, are manifested in some of the inoculated persons only after revaccination.

#### Discussion

As can be seen from this data on the investigation of inoculated

persons, vaccination, and especially revaccination, causes the formation of antihemagglutinins to all the representatives of the tick-borne encephalitis complex. However, regularity in the appearance of antibodies and their titers are subject to considerable fluctuation, depending on the titer of the virus strain being tested.

It is important to stress that the formation of antibodies took place with the same intensity to all the strains of tick-borne encephalitis virus under study regardless of the geographical region from which they were detected. Antibodies to the Omsk hemorrhagic fever and Langat virus strains under study were formed with almost the same constancy. Somewhat poorer results were noted when studying strains of the louping ill and Negishi viruses. Antibodies to the Kyasanur forest disease and Powassan virus strains used by us were detected only rarely and in low titers. Revaccination though caused an increase in the frequency of antibody appearance to these viruses, but to a lesser degree than to homologous virus and to the virus of Omsk hemorrhagic fever. In this respect our data are analogous to the results obtained by Pavri et al [7], who used the antigen of Kyasanur forest disease to investigate the sera of persons inoculated with vaccine against tick-borne encephalitis.

In our experiments there is a certain correlation between antihemagglutinating and virus neutralizing antibodies, and also between the latter and the development of resistance in vaccinated mice. Therefore, it can be assumed that the vaccine against tick-borne encephalitis is effective in respect to the tick-borne encephalitis virus not only in the eastern but in the western regions of its areal. Serological data from our experiments indicate the possibility of using the tick-borne encephalitis vaccine for the prophylaxis of diseases connected with the viruses of Omsk hemorrhagic fever and Langat. The possibility is not excluded of using this vaccine for immunization against the viruses of louping ill and Negishi. Together with this it is hardly justified to use our vaccine for creating immunity to the viruses of Kyasanur forest disease and Powassan. Inoculations with vaccine against tick-borne encephalitis in respect to the 3 last viruses can be expected to be effective only after one or several successive revaccinations. The problem of the possibility of creating a stable immunity to encephalitis requires an additional, more extensive, study of the regularities in the formation of resistance after the injection of group antigens. The presently known cases of laboratory infection with the viruses of Omsk hemorrhagic fever (in New York and in Omsk), and also the virus of Kyasanur forest disease (Puna, India), in persons who are strongly immunized with tick-borne encephalitis vaccine and who have antibodies to tick-borne encephalitis testify to the complexity of the problem.

Also the importance of the respiratory route of infection in laboratory cases has not been cleared up.

## Conclusions

1. In experiments for determining the resistance of immunized pureline mice and in investigations of the sera from inoculated volunteers in the neutralization reaction and the HI, a study was made of the immunogenic and antigenic properties of adsorbed tissue-culture vaccine from the tick-borne encephalitis virus in respect to various viruses from the subgroup of the tick-borne encephalitis antigen complex. Eight (8) strains of tick-borne encephalitis virus, isolated in the eastern and western regions of its areal, were used in the work, as well as the viruses of Omsk hemorrhagic fever, louping ill, Kyasanur forest disease, Langat, Negishi and Powassan.

2. The formation of antihemagglutinating and virus neutralizing antibodies in persons inoculated with adsorbed tissue-culture vaccine against tick-borne encephalitis takes place with the same intensity to all the strains of tick-borne encephalitis virus regardless of the region of their geographical distribution. Antibodies to the viruses of Omsk hemorrhagic fever and Langat are formed with almost the same constancy. Somewhat worse results are noted when studying the viruses of louping ill and Negishi. Antibodies to the viruses of Kyasanur forest disease and Powassan are detected rarely and in low titers. Revaccination though increases the frequency in the appearance of antibodies to the last two viruses, but it is far from sufficient. Close relationships are revealed when investigating the immunogenic properties of the vaccine.

3. The resulting data from the study of the antigenic and immunogenic properties of tissue-culture vaccine make it possible to assume that it is effective in respect to the tick-borne encephalitis virus not only in the eastern, but in the western regions of its areal. The results do not exclude the possibility of using tick-borne encephalitis vaccine for the prophylaxis of other infections, caused by viruses from the subgroup of the tick-borne encephalitis antigen complex. However, for a final solving of this problem it is necessary to have an extensive study of the regularities in the formation of immunological resistance after immunization with group antigens from the complex of viruses of the tick-borne encephalitis subgroup.

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Table 1

Strains of viruses from the tick-borne encephalitis antigen complex,  
used in the study of antibody spectrum

Virus	Strain	Information on isolation of the strain		
		Region	Year	Source
Tick-borne encephalitis	Sofin	Far East	1937	Man, central nervous system
	Khabarovskiy -17	Khabarovskiy Kray	1957	Same
	Bars	Western Siberia	1961	Man, clinically healthy, blood
	Alshevskiy	Western Siberia	1961	Man, central nervous system
	Pan	Moscow	1957	Man, laboratory infection, blood
	No 256	Belorussiya	1940	Ixodes ricinus
	Khpr	Czechoslovakia	1954	Man, blood
Louping ill	No 20536	Sweden	1961	Same
Omsk hemorrhagic fever	I-40	Scotland	?	Ixodes ricinus
Langat	Nikitina	Western Siberia	1948	Man, blood
	TP-21	Malaya	1956	Ixodes granulatus
Negishi	Negishi	Japan	1948	Man, cerebrospinal fluid
Kyasanur forest disease	W-372	India	1957	Monkey, blood
Powassan	Powassan	Canada	1958	Man, central nervous system

Table 2

Immunogenic properties of tissue-culture vaccine in respect to viruses from the tick-borne encephalitis complex (in experiments for testing resistance on mice).

Viruses Titers in 1g LD <sub>50</sub>	Khabarovskiy -17	No 256	Khpr	No 20536	Louping ill	Omsk hemor. fever	Langat	Negishi	Kyasanur forest disease	Povas- san
Non-immune mice	8.8	9.0	8.0	8.2	5.0	8.1	6.8	6.7	6.5	7.8
Immune mice	2.5	4.8	2.5	3.0	3.3	3.9	3.5	4.0	4.2	3.8
Indices of resistance	6.3	4.2	5.5	5.2	1.7	4.2	3.3	2.7	2.3	2.9

Table 3

Number of sera from inoculated persons examined in the III with 14 antigens from the tick-borne encephalitis complex

Period of examination	Antigens													
	Sofin	Khabarovskiy -17	Burs	Alshevskiy	Pan	No 256	Khlypr	No 20536	Louping ill	Omsk hemor. fever	Langat	Negishi	Kyzasunur Forest	Konassan
Prior to vaccination	46	46	1	1	10	1	10	1	1	1	10	1	10	10
After vaccination:														
10 days	46	34	34	31	33	34	31	34	34	33	46	38	33	31
30 days	9	10	11	10	10	10	9	10	7	10	10	6	10	10
Prior to revaccination	15	15	15	4	15	6	15	6	3	6	15	4	15	15
After revaccination:														
10 days	19	18	17	20	16	20	18	20	23	30	18	29	25	37
30 days	20	20	20	14	20	14	17	14	18	20	20	13	20	0
Total sera examined	155	143	98	80	104	85	103	85	86	105	119	91	113	106

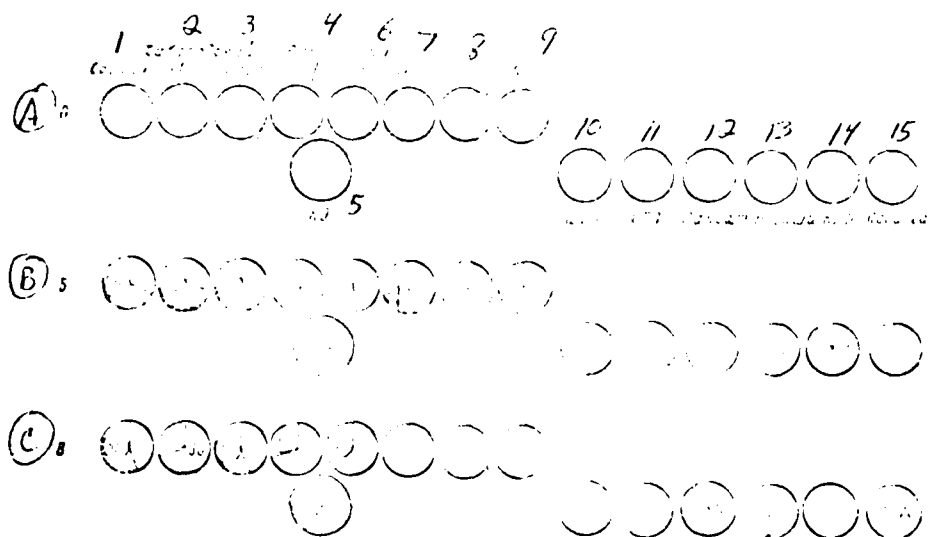


Figure 1. Percentage of positive results in the HI with antigens of the tick-borne encephalitis complex by investigating the sera of vaccinated men.

A - prior to vaccination;  
 B - after 10 days;  
 C - after 30 days after vaccination.

- 1 - Sofin
- 2 - Khabarovskiy-17
- 3 - Bars
- 4 - Alshevskiy
- 5 - Tick-borne encephalitis
- 6 - Pan
- 7 - No 256
- 8 - Khopr
- 9 - No 20536
- 10 - Louping ill
- 11 - Omsk hemorrhagic fever
- 12 - Langat
- 13 - Negishi
- 14 - Kyasanur forest disease
- 15 - Powassan

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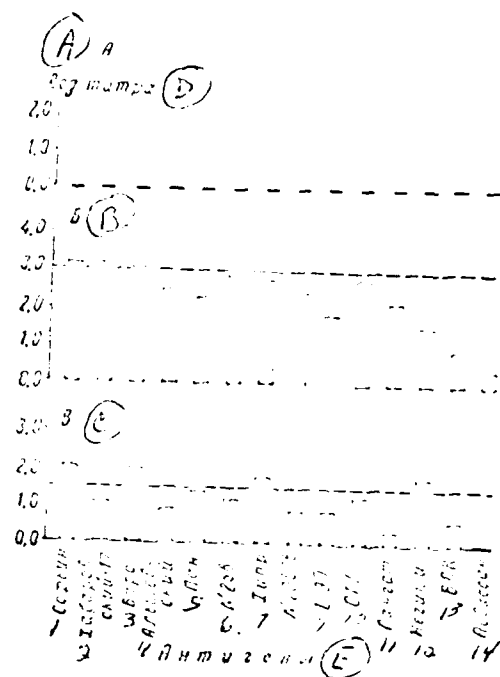


Figure 2. Titers of antihemagglutinins to viruses of the tick-borne encephalitis complex in man after vaccination.

A - prior to vaccination;  
 B - after 10 days;  
 C - after 30 days after vaccination;  
 D - log of titer;  
 E - antigens.

- 1 - Sofin
- 2 - Khabarovskiy-17
- 3 - Bars
- 4 - Alshevskiy
- 5 - Pan
- 6 - No 256
- 7 - Khopr
- 8 - No 20536
- 9 - Louping ill
- 10 - Omsk hemorrhagic fever
- 11 - Langat
- 12 - Negishi
- 13 - Kyasanur forest disease
- 14 - Powassan

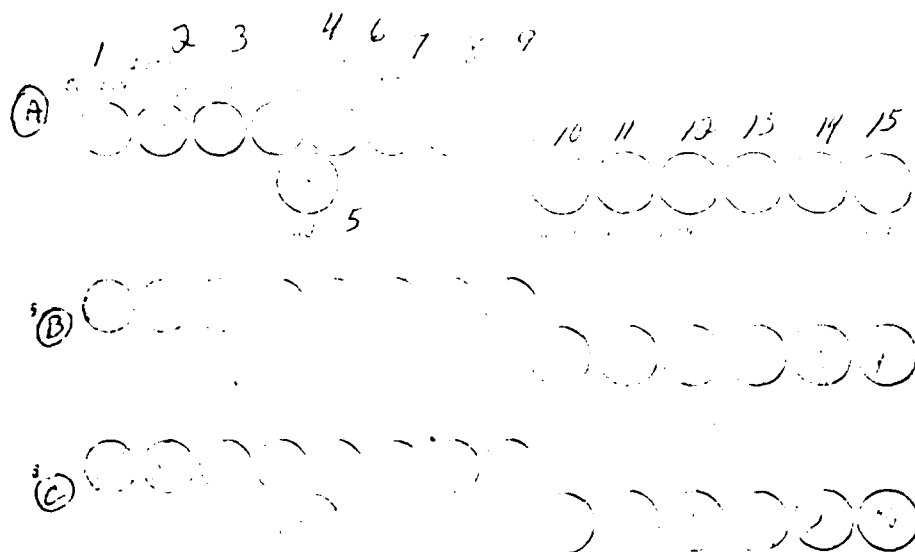


Figure 3. Percentage of positive results in the HI with antigens of the tick-borne encephalitis complex by investigating the sera of revaccinated men.

A - prior to revaccination;  
 B - after 10 days;  
 C - after 30 days after revaccination.

- 1 - Sofin
- 2 - Khabarovskiy-17
- 3 - Bars
- 4 - Alshevskiy
- 5 - Tick-borne encephalitis
- 6 - Pan
- 7 - No 256
- 8 - Khipr
- 9 - No 20536
- 10 - Louping ill
- 11 - Omsk hemorrhagic fever
- 12 - Langat
- 13 - Negishi
- 14 - Kyasanur forest disease
- 15 - Powassan

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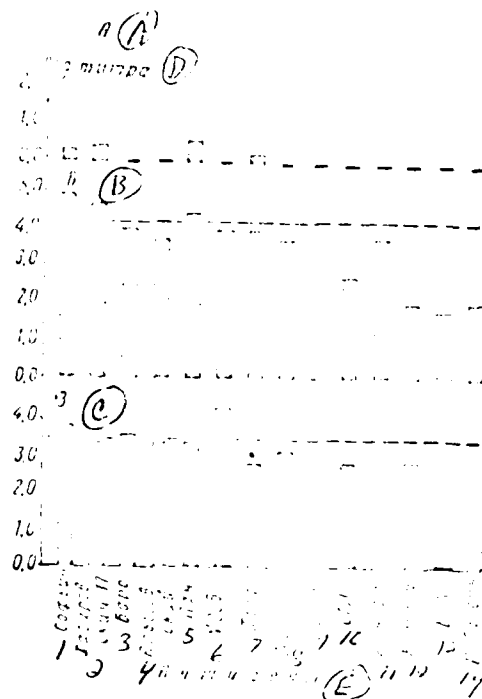


Figure 4. Titers of antihemagglutinins to viruses of the tick-borne encephalitis complex in man after revaccination.

- A - prior to revaccination;
- B - after 10 days;
- C - after 30 days after revaccination;
- D - log of titer;
- E - antigens.

- 1 - Sofin
- 2 - Khabarovskiy-17
- 3 - Bars
- 4 - Alshevskiy
- 5 - Pan
- 6 - No 256
- 7 - Khopr
- 8 - No 20536
- 9 - Louping ill
- 10 - Omsk hemorrhagic fever
- 11 - Langat
- 12 - Negishi
- 13 - Kyasanur forest disease
- 14 - Powassan

Table 4

Virus neutralizing activity in the sera of persons inoculated with tissue-culture vaccine in respect to viruses of the tick-borne encephalitis complex.

Viruses		Pan	No 256	Khpr	No 20536	Louping ill	Omsk hemor. fever	*Langat	Negishi	Kyasanur forest	Povassan
Sera	After vaccination	6	6	6	6	6	7	7	7	7	6
	Investigated Control (with Khabarovskiy-17)	2.4	2.6	2.8	2.7	2.7	2.6	2.7	2.8	2.6	2.7
	Test (with the given strain) Difference between control and test	2.3	2.7	2.6	2.9	2.1	2.2	2.3	2.4	1.8	1.1
After re-vaccination	Investigated Control (with Khabarovskiy-17)	-0.1	+0.1	-0.2	+0.2	-0.6	-0.4	-0.4	-0.4	-0.8	-1.6
	Test (with the given strain)	6	6	6	6	6	17	17	17	17	6
	Difference between control and test	4.0	3.5	4.1	4.0	4.3	3.7	3.8	3.8	3.8	4.1
	Investigated Control (with Khabarovskiy-17)	3.7	3.5	3.3	3.8	3.2	3.3	2.8	2.7	2.4	2.3
	Test (with the given strain)	-0.3	0.0	-0.3	-0.2	-1.1	-0.4	-1.0	-1.1	-1.4	-1.8
	Difference between control and test										

\* Intracerebral titration